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Aedestech Mosquito Home System Prevents the Hatch of *Aedes* Mosquito Eggs and Reduces its Population

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ABSTRACT

Dengue fever (DF) is a global health problem and considered to be endemic in Malaysia. Conventional mosquito traps currently applied as vector control do not effectively reduce *Aedes* mosquito population. AedesTech Mosquito Home System (AMHS) is an autocidal ovitraps for *Aedes* mosquitoes that uses the 'lure and kill' concept and is expected to be able to reduce *Aedes* mosquito population. The effectiveness of AMHS in reducing *Aedes* mosquito population was investigated in Block A, B and D (control) of the 17th College, Universiti Putra Malaysia (UPM). For the first two weeks (pre-intervention), the conventional ovitraps were used to obtain the initial abundance of mosquito population in Block A, B and D. Subsequently, AMHS was used for the next three months and again followed by the conventional ovitrap for the final two weeks (post-intervention). Ovitrap Index, Hatching Index and percentage of emergence of adult mosquitoes were calculated once every two weeks. Data were analysed using Paired Sample T-test. Values were considered significant at $p \leq 0.05$. The Ovitrap Index that indicates the mosquito population at Block A and B was significantly higher ($p \leq 0.05$) than of Block D. Hatching Index of AMHS was significantly lower ($p \leq 0.05$) then conventional ovitraps. All mosquito eggs collected in AMHS did not develop into adult mosquitoes. There was a significant reduction ($p \leq 0.05$) in the mosquito population between the pre- and post-intervention. In conclusion, AMHS

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was effective in reducing the mosquito population in 17th College, UPM. Therefore, it is believed to be a very promising vector management option to control the incidence of DF.

Keywords: Aedes, dengue fever, hatching index, ovitrap index, ovitraps, vector management

INTRODUCTION

Dengue fever (DF) or commonly known as dengue, is caused by dengue virus (DENV), which is a single-stranded RNA virus. DENV is a flavivirus from the family of Flaviviridae that is transmitted to an individual by a mosquito from an infected person. The mosquito acts as a vector for the virus. According to the World Health Organization (WHO), DENV causes a wide range of diseases in humans, from a self-limited DF to life-threatening syndromes called dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS) (WHO, 2017).

There are four DENV serotypes that can cause DF, which are DENV1, DENV2, DENV3 and DENV4. The presence of more than one serotype of DENV in a person's blood circulation indicates repeated infection of the virus leading to DHF and even DSS (CDC, 2017). The vectors that transmit DENV are mosquitoes specifically *Aedes* mosquitoes namely *Aedes albopictus* (Skuse) and *Ae. aegypti* (Linnaeus) (Rosen et al., 1983).

According to the Centre for Disease Control and Prevention, about 40% of the total population in the world live in areas with high risk of DENV transmission (CDC, 2017). An estimation of 50 to 100 million of the infection was deduced by the WHO in 2017. The fatality rate of DF was almost 25% of the reported cases (WHO, 2016). In Malaysia, the number of DF cases remains increasing. The total DF cases reported in 2018 was 80615, with 147 dengue-related deaths. The state of Selangor with 45349 DF cases recorded the highest number among all states in Malaysia. The first three months of the year 2019 recorded 37027 DF cases with 59 dengue-related deaths (iDengue, 2019).

The symptoms of DF can vary from a normal fever to a very serious convulsion. The general symptoms of DF include high fever, headache, vomiting, rashes, and joint and muscle pain (Tiga et al., 2016). There are also several warning signs, for instances, persistent diarrhoea, mucosal bleeding and serious decrease in platelet count. Immediate treatment is needed once diagnosed with DF as it could be fatal if there are severe plasma leakage, severe haemorrhage and severe organ impairment (WHO, 2009).

There are several approaches to prevent and control the DENV transmission including physical, biological and chemical control, and integrated vector management. Nevertheless, the number of DF cases is still increasing in Malaysia and globally although preventive and control measures are taken into action (Rajapakse et al., 2012). This is because the actions are not fully effective and infeasible to be applied everywhere.

The drawback of currently available ovitraps are that they are mostly unable to prevent the hatching of the eggs. Close and constant monitoring is required to avoid the development of adult mosquito from the eggs (WHO, 2009). This in turn can be very laborious and costly. Other vector control measure such as fogging or space-spraying with insecticide is only able to eliminate adult mosquitoes and not mosquito eggs (Chua et al., 2005). Recently, a vaccine for DF, Dengvaxia, has been developed. Nevertheless, it causes side effects to who have not been previously infected with DENV (Halstead, 2017). On that note, a more effective solution to curb the spread of DENV is crucial.

AedesTech Mosquito Home System (AMHS) is a commercial *Aedes* trap manufactured by One Team Network Sdn. Bhd. This invention emerged as the sole winner of Prevention-Vector Control group in the Dengue Tech Challenge 2016 funded by Newton-Ungku Omar Fund and British Council. AHMS works in the concept of "lure and kill", as it contains mosquito attractant which attracts adult female *Aedes* mosquitoes to lay eggs inside the trap (Lim Chee Hwa, personal communication April 29, 2018).

The attractant also contains an insect growth regulator (IGR), pyriproxyfen that effects the development of mosquitoes at various phases of its life cycles. The high ovicidal activity of pyriproxyfen at a dose as low as 1 ppm of multiple *Aedes* species has been documented (Suman et al., 2013). Due to the higher permeability of chorionic membranes of newly deposited eggs, pyriproxyfen has better ovicidal effects on those eggs than fully embryonated eggs of non-diapausing eggs. Pyriproxyfen on the other hand terminates egg diapause and are able to chemically induce the hatching of eggs from *Ae. albopictus* under diapausing conditions (Suman et al., 2015). Pyriproxyfen is also species specific as the ovicidal effect was effective against *Ae. albopictus* and *Ae. aegypti* but had no effect on *Ae. atropalpus*. Pyriproxyfen inhibits juvenile hormones and ecdysteroid titres of insects inhibiting embryogenesis and adult formation. Pyriproxyfen causes lowered fertility and fecundity in adults that develop from larva that has been exposed at sublethal levels of this IGR (Loh & Yap, 1989). Even though the pyriproxyfen is an effective larvicide and pupacide, it is not an adulticide.

On the basis that this trap is able to attract the deposition of eggs and prevents the development of eggs, this new intervention could be an effective method in vector management. This study investigated the effectiveness of AHMS in reducing the mosquito population in 17th College, Universiti Putra Malaysia (UPM).

MATERIALS AND METHODS

Study Site

Seventeenth College (17th College), one of the hostels in Universiti Putra Malaysia, UPM, was selected as the study area. The study area covers an area of 92, 015.75 m^2 with a population of approximately 1800 people. The hostel consists of four wings with five levels in each wing. Several student utilities are located within the block such as store, recreational room and study room. Forest and lakes can be found at the surrounding campus. There was abundance of breeding sites for *Aedes* mosquitoes found in this area especially around the big trees, fish pond, various types of artificial containers, drains and toilets. Block A and B were used for the intervention, whereas Block D was chosen as the experimental control for this study.

Conventional Ovitraps

Conventional ovitraps are dark containers that were prepared by using plastic drinking cups (opening diameter 7.8 cm, base diameter 6.5 cm and height 9.0 cm) painted in black. One oviposition substrate (10 cm x 2.5 cm x 0.3 cm) was placed in each ovitraps. The containers were filled with water and substrate to a height of 5.5 cm. The ovitraps mimics the dark breeding site of the mosquito, whereas the substrate is the surface for the mosquito to lay eggs. The traps were labelled according to the block, wing, and level they were placed.

AedesTech Mosquito Home System

The commercial *Aedes* trap (AMHS), was kindly supplied by One Team Network Sdn. Bhd. C-fold white paper towels (20 cm x 7.5 cm, Brand: Scott, Philadelphia) placed in AMHS, served as the surface for the mosquitoes to lay eggs. The traps were labelled according to the block, wing, and level they were placed.

Pre-Intervention Study

For the pre-intervention study, a total of 120 conventional ovitraps were placed at the corridors and along the staircases of Block A, B and D for the first two weeks. Two conventional ovitraps were placed at each level with a distance of less than five meters from each other and left there for four days. Wet substrates from the conventional ovitraps were collected (hereafter referred as sample collection 1) into a dry container and were replaced with new substrates. The wet substrates collected were brought back to the lab and dried, and the mosquito eggs attached to them were counted The Ovitrap Index was calculated using the following Equation 1.

$$Ovitrap\ Index = \frac{No. of\ positive\ ovitraps\ (with\ eggs)}{No. of\ ovitraps\ deployed} \times 100\% \qquad [1]$$

The substrates were then submerged in dechlorinated water for the eggs to hatch. The Hatching Index was then calculated using the following Equation 2.

$$Hatching\ Index = \frac{No. of\ eggs\ hatched}{Total\ no. egg\ counted} \times 100\% \qquad [2]$$

The species of larvae was then identified and killed using Dettol.

Intervention Study

For the intervention study, a total of 80 AMHS were placed at the corridors and along the staircases of Block A and B for the following three months. Two AMHS were placed at each level with a distance of less than five meters from each other. Wet tissues from the AMHS were collected by placing them into a dry container and replaced with new tissues for the next collection. The wet tissues collected were brought back to the lab and dried, and the mosquito eggs attached to them were counted. The Ovitrap Index and Hatching Index were then calculated.

Mid-Intervention Study

For the mid-intervention study, a total of 120 conventional ovitraps were placed at the corridors and along the staircases of Block A, B and D, after the first four weeks of the intervention study (hereafter referred as collection 2, 3, 4 and 5) alongside AMHS. The Ovitrap Index and Hatching Index were then calculated.

Post-Intervention Study

For the post-intervention study a total of 120 conventional ovitraps were placed at the corridors and along the staircases of Block A, B and D after the intervention study for two weeks (hereafter referred as collection 6). The Ovitrap Index and Hatching Index were then calculated.

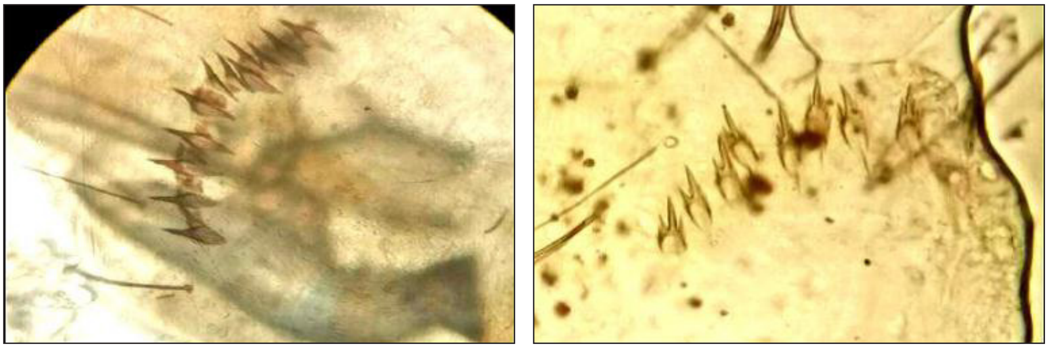
Mosquito Eggs Counting

The wet substrates in the conventional ovitraps or tissues in the AMHS were removed from the container. They were then dried using an oven at 50°C for one day. The substrate was sectioned into several parts to ease the counting of the eggs. The eggs were laid singly and black in colour (Figure 1). Next, the eggs attached on the substrate or tissue were manually counted under a dissecting microscope at ×20 magnification (Leica EZ4 Stereo Microscope, USA) and recorded.

Figure 1. Eggs of the Aedes mosquitoes attached on the tissue collected from AMHS as observed under a stereo-microscope

Species Identification

The dried substrates in the conventional ovitraps or tissues in the AMHS with the eggs attached were submerged in a container containing dechlorinated water or tap water, and left for four days. The number of larvae found in the container was counted starting from Day 5. The larvae were then collected one by one, placed onto a glass slide, covered with a cover slip, and observed under a compound microscope under the magnification of 400X (Leica, Germany). Species of the larva was identified based on the comb teeth structure in the larvae with reference to Farajollahi and Price (2013). The comb teeth of *Ae. albopictus* is thorn like (Figure 2a), while the one of *Ae. aegypti* is pitch-fork like (Figure 2b).



(a) (b)

Figure 2. Larva of (a) *A. albopictus* and (b) *A. aegypti* observed under a light microscope (400× magnification)

Data Analysis

Data were analysed using SPSS version 11.5. The difference between the pre- and post-intervention data was compared among Block A, B and D using the Paired Sample T-test. A value was considered significant at $p \leq 0.05$.

RESULTS

The presence of *Aedes* mosquito and its dominant species were screened using conventional ovitraps whereby the population of *Ae. albopictus* was significantly higher than *Ae. aegypti* (Figure 3). The effectiveness of AMHS in attracting *Aedes* mosquitoes to deposit the eggs were evaluated against conventional ovitrap. AMHS recorded significantly higher ($p \leq 0.05$) Ovitrap Index compared to conventional ovitrap (Figure 4). Furthermore, all the eggs present in AMHS did not hatch recording a hatching index of 0.00 ± 0.00%.

This value is significantly lower ($p \leq 0.05$) than the hatching index of the conventional ovitrap as shown in Figure 5. The hatched eggs from the conventional trap were allowed to develop into adult mosquitoes (Figure 6) recording a value of 93.13% and 92.85% in Block A and B, respectively. Since none of the eggs in AMHS hatched, the emergence of

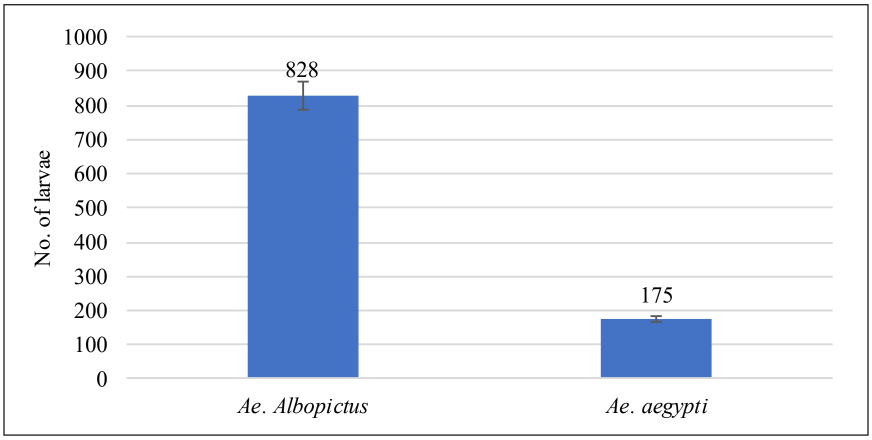


Figure 3. Number of larvae of *Ae. albopictus* and *Ae. aegypti* collected in the conventional ovitraps placed in Block A, B and D of 17th College, UPM

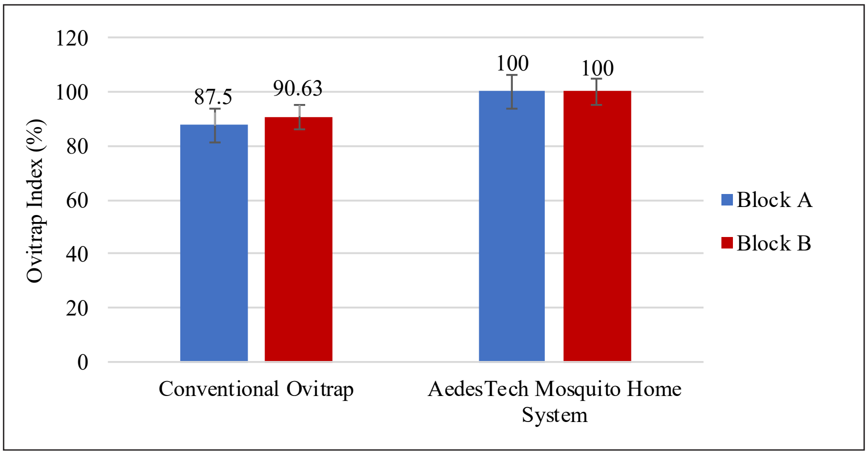


Figure 4. Ovitrap Index of the conventional ovitrap and AMHS in Block A and B of 17th College, UPM, during the pre-intervention study

adult mosquito was also 0% in both the blocks. The number of mosquito eggs collected in AMHS from each sample collection, as displayed in Figure 7, shows a general decreasing trend with drastic reduction between the first and second sample collections compared to the following collections. The reduction in the eggs count was significant ($p \leq 0.05$) after the three months of intervention using AMHS. The number of mosquito eggs in the three blocks where the study was carried out is represented in Figure 8. Prior to the intervention the mosquito population were almost similar among the three blocks. However, Block A and B installed with AMHS recorded a reduction during their mid- and post-intervention studies. Block D that served as the control remains with high eggs counts until the end of the study. Figure 9 depicts the OI in Blocks A, B and D during the pre-, mid- and post- intervention study. The blocks installed with AMHS show a decreasing trend in the number of traps positive with eggs but Block D shows and increase from 75 to 81.25% during the mid-intervention study before decreasing to 75% during the post-intervention study. The OI in the different levels of the 17th College building (Figure 10) shows the ground level and level 1 recorded the highest (83.33%) and lowest (66.66%) percentage of OI, respectively. No significant difference ($p<0.05$) in the OI was observed between the different levels of the building.

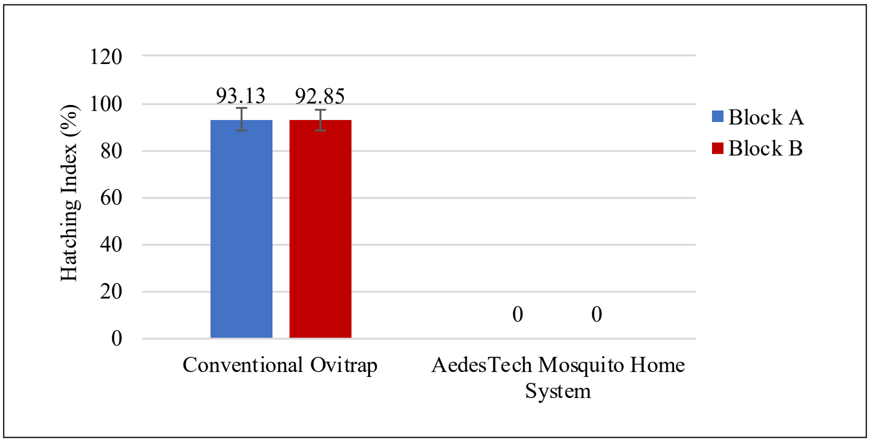


Figure 5. Hatching Index of the conventional ovitrap and AMHS in Block A and B of 17th College, UPM, during the intervention study

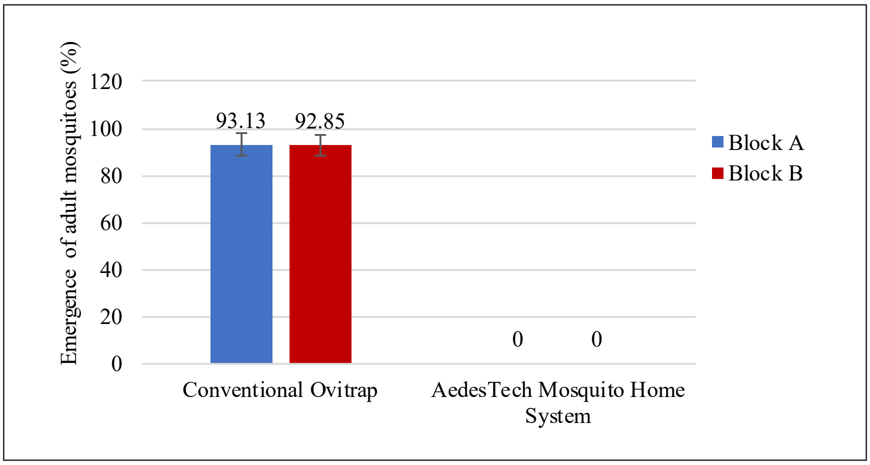


Figure 6. Emergence of adult mosquitoes from the hatched eggs of the conventional ovitrap and AMHS in Block A and B of 17th College, UPM, during the intervention study

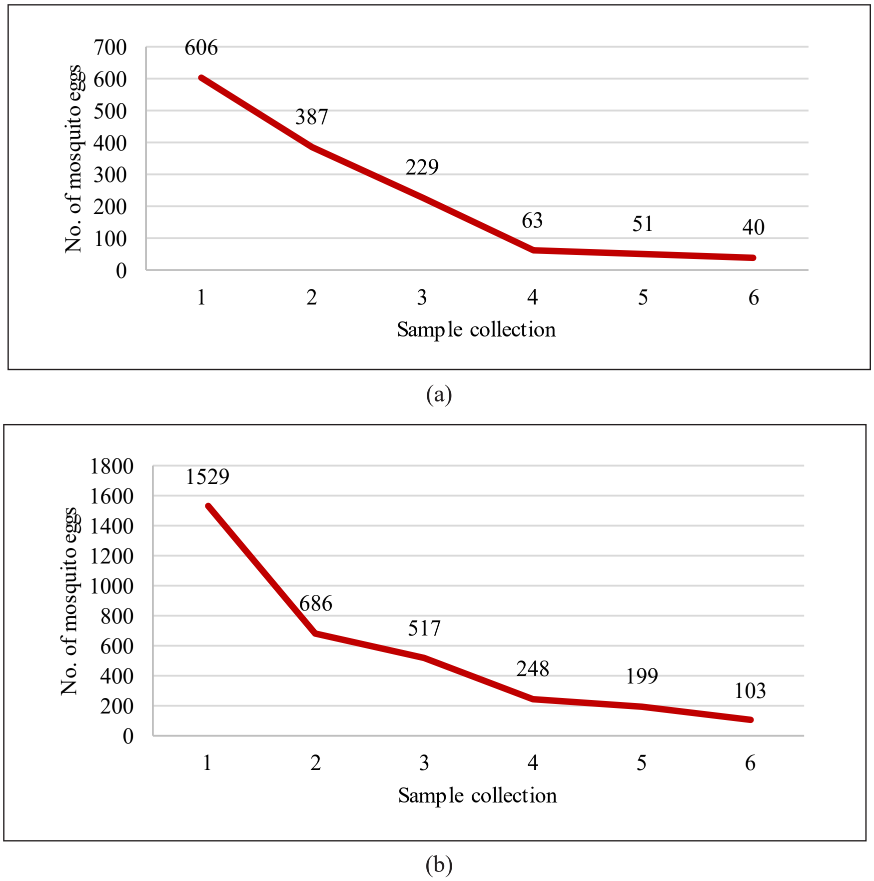


Figure 7. Number of *Aedes* mosquito eggs in Block A (a) and Block B (b) of 17th College, UPM, in the intervention study

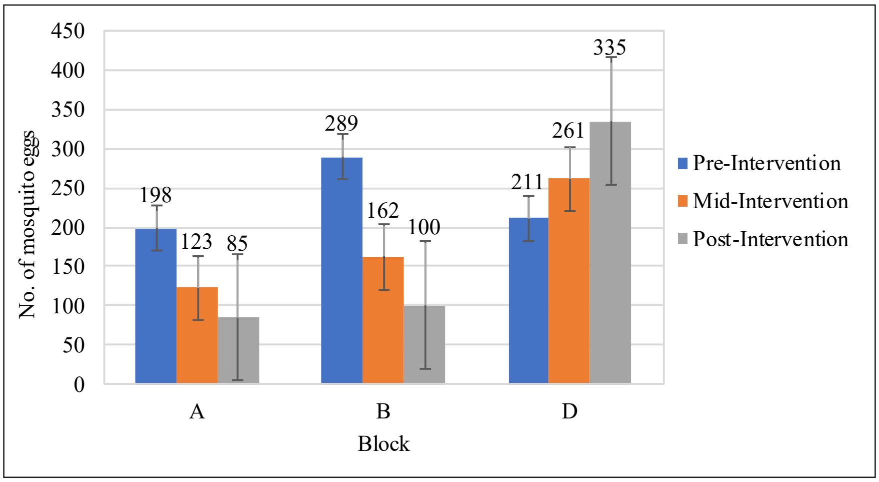


Figure 8. Number of *Aedes* mosquito eggs during pre-, mid- and post-intervention study in Block A, B and D of 17th College, UPM

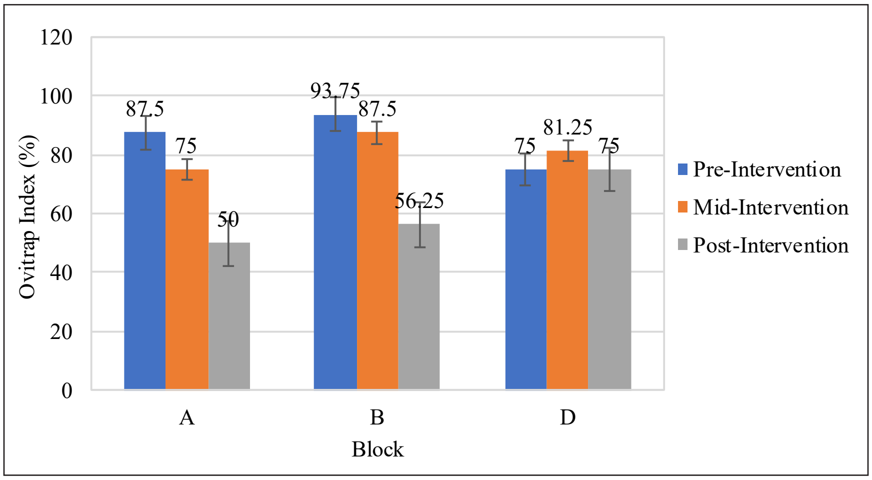


Figure 9. Ovitrap index during pre-, mid- and post-intervention study in Block A, B and D of 17th College, UPM

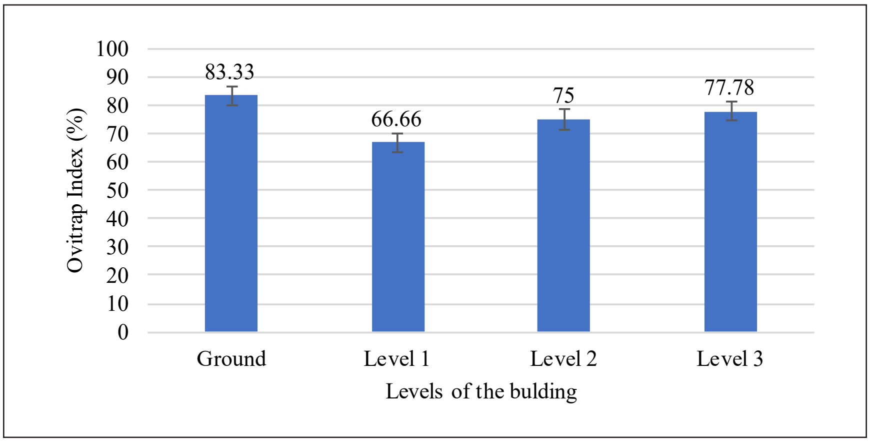


Figure 10. Ovitrap index in the different levels of the building in 17th College, UPM

DISCUSSION

There were many approaches studied in addressing the problems faced to reduce the DF cases (Achee et al., 2015; Bowman et al., 2016) but none is yet deemed effective. AMHS is a promising integrated vector management that attracts adult female *Aedes* mosquitoes to lay eggs in the trap and prevents the development of larvae from the hatched eggs. In this study, the effectiveness of AMHS to reduce the mosquito population was investigated in 17th College, UPM.

It was discovered in this study that *Ae. albopictus* and *Ae. aegypti* were the only *Aedes* mosquito species found in 17th College, UPM. The eggs were laid singly (University of Nebraska-Lincoln, 2018) and the uniqueness of *Aedes* larvae is that the comb scale structure is arranged in a single row (Barraud, 1934; Bar & Andrew 2013; Rueda, 2004). The college is surrounded with trees and lakes. *Ae. albopictus* and *Ae. aegypti* are commonly found and breed outdoor (in the forest and adapting itself to suburban and human environment) and indoor, respectively (MOH, 2017). *Ae. albopictus* was more dominant in number compared to *Ae. aegypti. Ae. albopictus* is associated with the term container breeder where it breeds in small containers found outdoor (Rozilawati et al., 2015) while adults of *Ae. aegypti* typically resides inside houses. Since this study was carried out by placing the ovitraps outdoor, that might be the reason that *Ae. albopictus* outnumbered *Ae. aegypti* in this area.

In this study, the eggs collected from the ovitraps hatched into larvae gradually starting from Day 4 (~10%) upon submerging in dechlorinated water, which was used to support bacterial growth that serves as food for the developing larvae. The presence of chlorine in water can kill the bacteria, hence, the developing larvae (Barrera et al., 2004). Most of the eggs (~75%) hatched in between Day 8 and Day 10. The average Hatching Index reached 82.83% to 93.13% from Day 10 to Day 14 (unpublished data). Similar findings were reported by the Centre for Disease Control and Prevention (CDC, 2017).

The effectiveness of AMHS to attract *Aedes* mosquitoes in comparison to the conventional ovitrap was evaluated based on the Ovitrap Index. Based on the higher Ovitrap Index of AMHS (100%) compared to the conventional ovitraps (90.63%), the concept of "lure" of the former worked in this study. AMHS contains attractant that attracts the female *Aedes* mosquitoes to lay eggs inside the trap (Lim Chee Hwa, personal communication April 29, 2018).

The Hatching Index was determined based on the number of larvae being hatched from the eggs, which is possible to be performed in the ovitrap because the larvae can be counted. AMHS contains pyriproxyfen that mimics a natural hormone that functions as an insect growth regulator, which inhibits development of adult mosquitoes from the hatched eggs (Hallman et al., 2015) by suppressing embryogenesis (Ishaaya & Horowitz, 1995). Similar finding was reported by Unlu et al. (2017), stating that the usage of pyriproxyfen showed significant reduction of larval population and also eggs count.

During the mid-intervention, the conventional ovitrap was used alongside with AMHS. There was no larvae development from the hatched eggs, hence, zero emergence of adult mosquitoes from the conventional ovitrap where the larvae are supposed to develop. It is postulated that the mosquitoes that laid eggs inside AMHS transfer pyriproxyfen in the solution via horizontal transfer to the conventional ovitrap, preventing the development of the larvae from the hatched eggs and later to adult mosquitoes. The tarsal and legs of ovipositing mosquitoes are exposed to the pyriproxyfen from the solution. The toxicant is able to attach and retain on the contaminated female and when the mosquitoes fly to another oviposition site, larvicide on their bodies may be transferred at a lethal concentration into the water killing existing larvae (Ohba et al., 2013; Wang et al., 2014).

There was a significant reduction in the number of mosquito eggs between collection 2, 3 and 4 performed for 6 weeks (mid-intervention) using both conventional ovitrap and AMHS. Drastic reduction (by ~50%) was noted between the first and the second collection. The reduction was less drastic between the second and the third collection (by ~20%). It is postulated that AMHS has prevented the emergence of adult mosquitoes in the second collection that the mosquito population is reduced following the third and fourth collection. Hence, the mosquito population kept on reducing as the weeks went by for three months.

The control group, which was Block D, showed an increase in the *Aedes* mosquito eggs count in contrast to the reduction of eggs in both Block A and B. This shows that it is the use of AMHS which has reduced the mosquito population, not the external interference or environmental factors such as fogging and the change in weather that might have caused the reduction of mosquito population in Block A and B. Furthermore, the outcome of OI also support this claim whereby Block D with no intervention showed no reduction in the number of ovitraps positive with eggs but was clearly observed in Block A and B. The possible risk of an outbreak to occur in a particular area is indicated by OI value of more than 10% (Braks et al., 2003; MOH, 1997). We recorded above 50% of OI even in Blocks A and B indicating that even though AMHS was able to reduce the mosquito population, it is insufficient to fully eliminate the risk of possible outbreak in the vicinity of 17th College, UPM. Moving forward, efforts to improvise the intervention or increase the number of traps could be carried out to reduce the mosquito population more effectively.

Ovitraps were deployed in all the four levels (ground level, level 1, level 2 and level 3) of the hostel building and OI was not affected by the different elevation of the building. This outcome is similar to the reports by Lau et al. (2013) in a study carried out in multiple storey buildings in four residential areas located in Selangor and Roslan et al. (2013) who conducted the experiment in apartments in Kuala Lumpur, Malaysia. However, the study by Wan-Norafikah et al. (2010) reported that lower *Aedes* sp populations was found at higher levels of high-rise apartments. That outcome was different to ours probably due to the different structure of the buildings where both these experiments were carried out. High-rise buildings are buildings with more than five stories while buildings fewer than five stories and landed properties are considered low-rise buildings (Mahmud et al., 2018). Since the building in our study area falls within the category of low-rise building, the mosquito population was not affected by the elevation.

Data from this current study are in concordance with previous report whereby, researchers have correlated the density of adult mosquitoes with the number of eggs being collected in the ovitrap. Higher number of eggs indicates higher density of adult mosquitoes. The reduction of the eggs count reflects the reduction of the mosquito population (Reiter et al., 1991).

CONCLUSION

The 17th College, Universiti Putra Malaysia, has high population of both species of *Aedes* mosquitoes namely *Ae. albopictus* and *Ae. aegypti* with the former being more dominant. The commercial *Aedes* trap, AedesTech Mosquito Home System, was effective in reducing the mosquito population in the college.

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